A

Synapsis

On

"Studies on Chemical and Biological control of leaf spot disease of *Tinospora cordifolia* caused by *colletotrichum spp.*"

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"Studies on Chemical and Biological control of leaf spot disease of *Tinospora cordifolia* caused by *colletotrichum spp."*

1. INTRODUCTION

The family Menispermaceae is in the major group of Angiosperms, viz,. commonly known as the moonseed family. It consist of approximately 70 genera and 450 species distributed mainly throughout the tropical and subtropical region in the world (Wang et al, 2007and Meng et al, 2012).

All the members of the family are unisexual and dioecious. Most species of Menispermaceae contain bioactive compounds, such as alkaloids, flavonoids, lignans, steroids, terpenoids, and coumarins. (Semwal et al, 2010). In India, it has 43 species and 20 genera. The family menispermaceae is highly specialized in its rich bisbenzyliso quinoline alkaloids. This family is used worldwide in traditional medicine to treat a variety of ailments. (Barbosa – Filho et al, 2000 : De wet et al, 2004 and De wet et al, 2005).

A literature survey revealed that twenty one genera are used for medicinal purposes in Africa and Twenty nine genera in the rest of the world. The following nine genera are being used in both Africa and the rest of the world. Out of nine genus Tinospora (menispermaceae) mostly used in medicine (Neuwinger, 2000 ; Duke, 2007and De wet, 2006).

As regard to detailed literature based biochemical changes in leaf due to leaf spot diseased caused by *Colletotrichum spp.* of *Tinospora cordifolia* will be elaborated in the main text of thesis.

Origin and geological distribution

Global distribution

Tinospora cordifolia is found in India, Myanmar,Shrilanka and Bangladesh.

Indian distribution

Tinospora cordifolia is distributed throughout the tropical region of India upto 1200 m above sea level from Kumaon to Assam. In North extending through west Bengal, Bihar, Decan, Kokan, Uttar Pradesh, Kranataka, kerala, Odisha ,Sikkim, Goa, Assam, Arunachal Pradesh, Maharastra.

State distribution

In Maharashtra, it commonly found everywhere including Marathwada region.

Morphological characters of *Tinospora cordifolia* **(Wild) Miers**

Tinospora cordifolia (Wild) Miers belonging the to family Manispermaceae of order Ranunculales, common name Guduchi(Sanskrit), Local name Giloy(Hindi), other names Gurue, Gllow, Gulvel(Marathi).

It is known as heart leaved moonseed plant. It is a large deciduous extensively spreading climbing shrub with several elongated twining branches. The Plant is very rigid and it can be grown in almost all climates but prefers warm climate. Planting is usually done during rainy season (July to August). As it is climber, so it requires support for its fast growth species such as Neem (*Azadirachta indica*), Jatropha(*Jatropha curcas*) and moringa (*Moringa loeifera*) have been planted to provide support for its growth.

Tinospora cordifolia growing with Neem (*Azadirachta indica*) is called as Neem giloy has chemical composition shows better Therapeutic properties.*Tinospora cordifolia* prefers medium black to red soil for its cultivation Giloy can also be successfully grown in large variety of soils, ranging from sandy to clay loam.However, the soil should be well drained with sufficient moisture and rich with organic matter for its growth.(Mishra et al, 2014). The detailed morphology is as follow.

Stem- Fibrous and the bicollatoral vascular budndles surrounded by pericycle succulent with long filiform fleshy and climbing in nature.

Bark- Creamy white to grey brown warty deeply left spirally the space in between being spotted with large rosette like lenticels.

Root- Succulent with long filiform fleshy aerial roots from the branches cork arises in the subepidermal layers and gives rise to 2-3 layers of cork, starch is present throughout the parenchyma of the stem. Aerial root, are present. These aerial roots are characterized by tetra to penta arch primary structure and starch is present throughout the parenchyma of aerial root.

Leaves- Simple, alternate, stipulated, cordate, long, petioles upto 15cm long, roundish, pulvinate, both at the base and apex with basel one longer and twisted partially and half way round.

Lamina- Ovate or ovate cordate 10-20 cm long,8-15cm broad, 7 nerved and deeply cordate base membranous, whitish tomentose with prominent reticulum beneath.

Flowers- Unisexual small on separate plants and appearing when plant is leafless , greenish yellow on axillary and terminal racemes male flower clustered and female during summer.

Sepals-6 free in two series of three each, the outer one are smaller than inner petals, 6 free smaller than sepals, obovate and membranous.

Petals- 6 free smaller than sepals obovate and membranous.

Fruits- Aggregates of 1-3 ovoid, smooth druplets on thick stalk with sub terminal style scars, scarlet or orange red colous fruits grow during winter.

Seeds- White bean shaped curved.

Cultivation - *Tinospora cordifolia* is cultivated by using of stem. It grows well in almost any type of soils and under varying climatic conditions. The stem cutting in the month of May – June support preferably Neem and Mango trees, such plants are supposed to possess better medicinal values.

Periodical hoeing is done both in the Nursery and field as per requirement. The medicinal plants have to be grown without the chemical fertilizers. Organic mannures like farm yard mannure [FYM], vermi compost, Green mannure etc. may be used as per requirement of the species.

To prevent diseases, bio-pesticides could be prepared (either single or mixture) from Neem (Karnel seeds and leaves) chitrakmol, dhatura, cow's urine. The field after plantation should be irrigated periodically as and when required weekly or fortnightly mature plants are collected, cut into small pieces and dried in shad, yield approximately 8-10 g/ha.

Area under cultivation

Large scale cultivation in Nasik, Miraj, Kolhapur Ayurvedic ras shala cultivating on large scale and small scale cultivation of *Tinospora cordifolia* is for the purpose of making drugs for marketing.

In Patanjali Ayurvedic Research centre *Tinospora cordifolia* is also cultivating on large scale for the medicinal purpose.

Species of Tinospora

1] *Tinospora crispa Miers*

2] *Tinospora malabarica* (Lam) Miers

3] *Tinospora tomentosa* (cole br.) Miers

4] *Tinospora cordifolia* (Willd) Miers

History of *Tinospora cordifolia*

The ayurvedic drug Guduchi or Amrita is mentioned in various classical texts of Ayurvedic system of medicine viz. Charak, Sushrut, Ashtana Hridaya and other treaties like Bhava Prakash and Dhanvantari Nighanu. Other various names such as Amara, Amritvalli, Chinnaryhuha, Chinnodebha.

In Sushruta samhita, it is mentioned under "Tikta saka varga" and claimed to be useful in treating kustha [Leprosy], maha – jvara [kind of fever], svasa [Asthama], Aruchi [anorexia], charak samhita, Ashtana Hridaya it has been indicated in diseases like kamala [Jaundice], Jvara [Fever], vat Rakta [gout].

In Bhava Prakash, it is considered as a bitter tonic astringent diuretic and potent aphrodisiac, and curative against skin infections, Jaundice, diabetes, and chronic diarrhoea and dysentery.

In Dhanvantari Nighantu, it's medicinal properties are mentioned for cure of bleeding piles, promoting longevity, curing itching, erysipelas. It is reported to be potent vegetable tonic being a rejuvenator, It is indicated in several diseases causing debility.

The feeula is Nutritious especially in diarrhoea, largely given in cold fevers, seminal weakness in urinary affection, jaundice skin disease, various forms of diabetes and irritability of stomach.

It is a traditional belive among the Ayurvedic practictioners that Guduchi satva obtained from the Guduchi plant growing on Neem trees [*Azadirachta indica*] is more bitter and more efficacious and is said to encorporate the medicinal virtue of Neem also.

The drug also caught the attention of European physicians in India as specific tonic, antiperoidic and for it's diuretic properties and it was included in the Bengal pharmacopoeia of 1868.

Tinospora cordifolia is mentioned in Ayurvedic literature as a constituent of several compound formulations used in general debility dyspepsia fever and urinary diseases. Some of the important formulations are Guduchyadi churna, Guduchi taila, Dashmoolarishtha, Sanjivani Vati, Kantakari avaleha, Guduchi satva, Arnarita Guggulu.

In unani system mostly "Sat Giloe" is in corporate in the preparations.

Use in Folk and Tribal medicine

Tinospora cordifolia is specially mentioned for it's use in tribal or folk medicine in different parts of the country. Some of the important uses mentioned are given below. The tribals Baiga living in the interior areas of Naugarh and chakia Block of Varanasi district Uttar Pradesh makes the paste of stem of the Guduchi and the roots of

Bhatkatiaya [solanum surattense]. The pills are prepared and used in the treatment of fever for three days.

The tribals of Bombay and it's Neighbouring areas and the fisherman along the sea coast use *Tinospora cordifolia* drug in the treatment of fever, Jaundice, chronic diarrhea and dysentery.

The tribals of khed brahma region of North Gujrat use the plant in their day to day life as food medicine. They use powered root and the stem bark of *Tinospora cordifolia* with milk for the treatment of cancer, decoction of root is used for the cure of dysentery and diarrhea.

Decoction of old stems is preferred in the treatment of periodic fever, decoction of stem is administered orally by the people of Jammu and Bigwada (Rajasthan) for the treatment of fever.

The inhabitants of Bhuneshwar (Orrisa) use the warm juice of root of *Tinospora cordifolia* orally for treatment of fever.

The Muslim tribals of Rajouri, Jammu (Tawi) comprising Ggujjar and Backwals used the plant in bone fracture.

In Dahanu Forest division of Maharashtra tribal races, viz. Agaris, Bhils, Dhodias.

Paste or Juice of Amrita leaves and sarsapa beeja churna [seed powder of Brassica campestris] is applied locally in case of Daha [Burning sensation].

Two drops of leaf Juice of allied species of Guduchi is dropped in the ear for the treatment of karna shula [pain in ear] by the local people of Patiyala [Panjab].

Economic importance of *Tinospora cordifolia*

It plays a vital role as immune booster. It helps in promoting self defence mechanism of our body useful in treatment of Jaundice and related diseases of liver, It acts great in balancing body sugar level and also act as Antipyretic drug useful to relieve cough improves digestion powers. It helps in the management of anemia. It acts as antiviral. It have great role in management of infections. It helps as rasayan and delays the normal aging process.

It promotes the normal cell growth, it is rich source of antioxidants, works great in treatment of skin disorders. A very good drug for auto immune system diseases. Stem of *Tinospora cordifolia* is one of the constituents of several Ayurvedic preparation and used in general debility, dyspepsia, fever, urinary, stomachic diuretic stimulates bile secretion causes constipation allays thirst, burning sensation vomiting. (Bhattacharya chandrayee and Bhattacharya, Gautam(2013).

Different Ethnobotanical and pharmacological uses of *Tinospora cordifolia*

Leaf- Used in the treatment of gout and ulcer. Leaf extract showed inhibition of *E.coli* Extrac is of leaves improves fertility and decoction of leaves cures malarial fever.

Stem- Bitter, stomachic, stimulates bile secretion, diuretic enriches the blood, cures jaundice, useful in skin diseases, Juice is useful in diabetes, vaginal and urethral discharges, fevers and enlarged spleen.

Root- A powerful emetic, visceral obstruction, water extract is used in Leprosy.

Stem + Root- (Combined with other drugs as an antidote to snake bite)

Fruit- With ghee or honey used as tonic and in treatment of Jaundice and rheumatism juice of Ripe Fruits with a little honey is given to children in cold.

Diseases controlled :-

Allergic diseases, anemia, anorexia, asthma, cancer, chronic diarrhea and dysentery, diabetes mellitus, fever, gonorrhea, gout, heart diseases, helmenthiasis, inpotency, irritability of stomach, jaundice, leprosy, malaria, skin diseases rheumatoid arthreitis syphilis, tuberculosis, urinary diseases, viral hepatitis.

Biochemical constituents (Phytochemical)

Tinospora cordifolia belong to different classes of constituents such as alkaloids,diterpenoid, lactones, glycorides, steroids, phenolies, sesquiterpenoid, aliphatic compounds, polysaccharides.

Alkaloids in stem and root shows active principle with reference berberine, palmatine, tembetarine (0.012%), magnoflorine (0.075%), choline, tinosporin, isocolumbin, tetrahydropalamatine.

Glycosides in stem part shows active principle with 18 norclerodane – glucoside, furanoid diterpene glucoside. Tenocordiside, Tinocordifolioside, cordioside, cordifolioside A[27,28] cordifolioside B[27,28] syringin.

Diterpenoid lactones are occurs in whole plant body shows active principle with furanolactone, clerodane – derivatives, Tinospora, Tinosporides, Jateorine, columbin – steroids clero is occurs in aerial part stem with active principle b-sitosterol, d-sitosterol, 20b- hydroxy ecdysone, Ecdysterone mukisterone A, Giloinsterol, Sesquiterphenoid, aliphatic compounds are present in stem, whole plant with active principle tinocordifolin, octacosanol, heptacosanol, nonacosan.

Miscellaneous compounds occurs in Root, whole plant body with active principle. 3(a, 4dihydroxy – 3 methoxy – benxyl); 4(hydroxyl - 3 methoxy - benzyl); tetrahydrofuran, jattrorrhieine, tinosporidine, cordifol, cordifelione, gilon, tinosporic acid.Chemical constituents of leaf of *Tinospora cordifolia*. isolated essential oil and evaluation of it's in – vitro. Antioxidant activity is reported.

Essential oil was obtained by the hydrodistillation using Clevenger- apparatus was found to contains 27 volatile compounds by GCFID. And GC-MS analysis, identification of 25 constituents comprising – 98.1% of essential oil was done using NIST Library and wiley GC – MS library – alcohols (32.1%), phenols (16.6%), aldehydes (16.2 %), fatty acids (15.7%), alkanes (8.3%), esters (3.2%), terpenes (1.2%), other classes of compounds (4.8%) major components of the leaf essential oil were hydroquinone (16.6 %), 2 hexenal (14.2 %), palmitic acid (14.1 %), 2 hexen-1 (11.5 %), phytol (11.4 %).

2. Review of literature and development in subject

India is one of richest countries in the world with huge diversity of medicinal plants Chemicals present in the medicinal plants are known to have medicinal benefits i.e., referred to as "active ingredients" or "active principles".

Phytochemicals include terpenoids, phenolics, alkaloids and fiber research supporting beneficial roles for phytochemicals against cancers, coronary heart diseases, diabetes, high blood pressure, inflammation, microbial, viral and parasitic infections.

These phytochemicals are mainly synthesized in root, stem, bark, leaf, flower, fruits, medicinal plants are mainly screened for these phytochemicals that may lead to its further characterization of bioactive compound. Isolation of bioactive compound from plants is mainly depending upon the solvents which are used for extraction.

Phytochemical analysis of medicinal plants attracts the attention of plant researchers due to development of more sophisticated tools. These tools played an effective role in the search of resources of un analyzed materials for pharmaceutical research.

Somashekhara et al (2015) studied *Tinospora cordifolia* as an important medicinal plant used in the treatment of several diseases and disorders.

Gaur et al (2014) observed that *Tinospora cordifolia* found mainly in India, Malaysia, Srilanka. There are about 40 species of Gilloy which found through out the world. Comprising parts of Africa, Southern Eastern Asia and Australia. Out of 40 species, only 4 species have been found in India.

This significant plant is also mentioned in important pharmacopoeias (BPC 1994, IPC, Govt. of India 2007 and Dept. of AYUSH, Ministry of Health and Family Welfare,

Govt. of India 1999) *Tinospora cordifolia* called in different state, and different language Heart leaf moonseed (English), Gulvel (Marathi), Amruthaballi (Kannada). In ancient Indian literature, is plant is termed as Amrit (Sanskrit) and Abb-e-Huat (Urdu) meaning water of life, (Giloya) Hindi. [Dept. of Indian system of medicine and homeopathy,New Delhi Welfare 2001].

Hare et al, (2014) studied and identified fungal diseases on *Tinospora cordifolia* based on symptoms and pathogen nature, on leaf of *Tinospora cordifolia* causing leaf spot by *Alternaria alternata.*

Singh et al, (2012) observed the fungal diseases of *Tinospora cordifolia as* leaf spot, amphigenous, sub circular to irregular, blackish brown, scattered along the veins, up to 16 mm in diameter caused by *Pseudocercospora Tinospora* (Kamal et al, 2003 and Brawn 2000).

Ajrekar and Oza (1932) reported *Glomacrella Cingulata* on leaves. Mundkar (1938), Thirumalachur and chupp (1948) observed leaf spot due to *Cercospora tinospora*. Thirumalachur and Lacy (1951) leaf spot caused by *Phyllachora dolichospora*. Tilak and Kale (1970) found parasitic stem fungus due to *Ulecodothis indica*. Chandra (1974) studied leaf spot due to *Cercospora verruculosa*.

The plant is very hardly and it can be grown in almost all climates but prefers warm climate, black soil (vertisols) or red soil (Alfisols) found in Tamil Nadu is the best for the cultivation of *Tinospora cordifolia*. *Tinospora cordifolia* can be propagated by seeds and also vegetative cuttings, the best way is vegetative way. Hartmann et al, (1997). Cutting of *Tinospora cordifolia* will be ready for planting in to the main field by this time in the month of May – June. Flowers appear during the winter. Kirtikar and Basu, (1975) and Anonymous, (1976).

Desai et al, (2012) studied and observed phytochemical constituents of *Tinospora cordifolia* leads to the isolation and identification of a compound alkaloids, flavonoids, steroids, saponins, total phenols, cardiac glycosides and reducing sugars.

Chintalwaret et al, (1999) isolated three major groups of compounds, protoberberine, alkaloids, terpenoids and polysaccharides. i.e. considered as purative active constituents of *Tinospora cordifolia*.

Symptomatology

Phoma leveillei caused leaf spot disease in *Tinospora cordifolia, colletotrichum spp* are known to cause disease leaf spot (Perfect et al.,1999)

Naik et al. (1990) noticed that the incidence of infection by *c. gloeosporioides* on piper betel was maximum in agun.

Kuch (1990) reported bligut disease of piper longum caused by *colletotrichum capsici.*

Gupta et al. (1997) observed the leaf spot disease of cassi angustifolia. The causal of organism was reported as *C. gloeosporioides*.

Mc Rae (1917) reported *colletotrichum* leaf spot the first time from Coimbatore, Tamilnadu.

Butler and Bisby (1931) transferred the genus vermicularia to *colletotrichum.*

Joshi and Sharma (1980) reported *colletotrichum* leaf spot of turmeric from Madhya Pradesh.

Pathogenicity

Menon and Rama Devi (1967) proved- pathogenicity of *colletotrichum spp.* Inciting spot disease of nutmea

Sathyarajan and Naseema (1985) observed the leaf spot diease of piper longum caused by *colletotrichum gloeosporioides.* The pathogenicity was established by spray of the spores collected from 10 days old culture.

Gupta et al. (2007) observed some medicinal plants in Madhya Pradesh, India infected by different fungi. Among the observations were : *c. gloeosporides* on *Ipomea obscura ; colletotrichum caudatum* on *cymbopogan* martini ; *colletotrichum dematium* on *Aconus calamus.* The pathogenecity of all the fungi isolated was confirmed by inoculation of culture of the same on concerned original hosts.

Sharma and Verma (2007) isolated the fungus *colletotrichum gloeosporioides* from the anthracnose infected mango leaves.

Efficacy of different fungicides against *colletotrichum spp.*

Narain and Panigrahi (1971) showed that vitavax, plantvax and |Aureofungin were equally effective in reducing the infection.

Joshi and Wangikar (1978) found that cumin, Dithane Z-78, Dithane M-45, captaf, Bavistin, Ziride and Benlate completely inhibited the mycelia growth of *colletotrichum capsici.*

Rao and Rao (1987) observed that six sprays with Dithane M-45 @ 0.25% at an interval of 15 days reduced the disease.

Palarpawar and Ghurde (1989 b) reported Bavistin to be most effective in controlling the disease followed by topsin- M. Ninosan and Karathane.

Narasimhudu and Balasubramanian (2001) Found thiophanate methyl completely inhibited the growth of *colletotrichum capsici.*

Ali et al. (2002) reported that leaf spot incidence can be reduced by foliar spray of Bavistin.

Effect of different plant extract against *colletotrichum spp.*

Gupta et al. (1981) reported that germination of conidia of *colletotrichum capsici* was inhibited by phytonocides of Allium Cepar. A sativum L., *Azadiracta indica, ocimum basilicum* and *Leucas spp.*

Jetti et al. (1987) reported that leaf extracts of *polyanthia longifolia L.* inchibited the growth of *colletotrichum gloeosporioides.*

Johri et al., (1994) reported that saponins from Mimusaps elengi and M. littoralic seeds were 100% effective against C. capsici.

Shivapuri et al., (1997) noticed that A. Indica *Datura stranomium L. O sanctum L., P longifolia L., Vinca rosea L* were more fungitoxic against pathogen.

Gomathi and Kannobiran (2000) found that aqueous leaf extracts of *solanum torvum, D metel* and *prosopis juliflora* effectively inhibited the conidial germination and mycelia growth of C. Capsici.

Efficacy of different bioagents against *colletotrichum spp.*

Medeiros and menezes (1994) observed that *C. gloeosporioides* (G. cingulata) showed a high degree of sensitivity to bioagents viz, *T. harzianum, T. polysporum* and *T. pseudokoningi.*

Jeyalakshmi et al (1998) Screened fungal and bacterial antagonists against *C. Capsici* causing fruit rot and dieback of elvilli, The maximum growth inhibition of the pathogen was exerted by saccharomyces cerevisiae followed by *Trichoderma viride, T. hamatum, T. pileatus* and *T. harzianum.* Among the bacterial antagonists *Bacillus subtilis* showed the maximum growth reduction followed by *Pseudomonas Fluorescens* isolate 27.

Gud (2001) reported that *C. gloeosporioides* isolated from mango was effectively inhibited by *Trichoderma harzianum, T. viride, T. Koningii, T. lingnorum* and *gliocladium virens* under in vitro studies. The reduction in growth ranged between 29.02 to 77.07 %

Singh (1985) found *Epicoccum purpurescens* a non pathogenic epiphytic fungus to be highly antagonistic against *C. capsici.*

Gupta et al., (1991) observed inhibition of C. lindemuthianum on French bean by *Gliocladium* virens, *T. harzianum* and *T. viride* in vitro.

Jeyalakshmi et al. (1998) reported that *T. viride, T. harzianum and T. Koningii* inhibited mycelia growth of *C. Capsici.*

Diseases and their causal organism of *Tinospora cordifolia*. Like leaf spot disease and its effect on secondary metabolite production was studied by Manchanahally et al ; (2017).

The pathogens like *Alternaria alternata, Cadosporium cladosporioides, Pestalotia macrotricha, Phomaputaminum cpyenidia. Penecilium spp. (12.62%), Collectotrichum spp. (11.8%), Claclosporium spp. (8.9%), Chaetomium globosum. (8.1%), Cunllaria spp. (7.6%), Alternaria alternata (6.8%), Fusarium oxysporum, Aspergillus Flavus, Aspergillus tubingensis.* Were studied by Mishra et al, (2012) and Leaf spot on *Tinospora cordifolia* caused by *Alternaria alternata* and a new flat stem disease of *Tinospora cordifolia* caused by phytoplasma was found by Somashekhara et al, (2015).

Among these diseases leaf spot diseases results into heavy lossess of leaf therefore, selected for detailed investigation in relation to *colletotrichum spp.*

3. Objectives of the Research :

The objectives of this research is to evaluate potential biochemical changes due to leaf spot disease caused by *Colletotrichum spp.* on leaf of the *Tinospora cordifolia*. This is a dioecious plant. The male and female plants are different. The plant contains more phytochemicals. Hence selected for detailed investigation. This work will be carried out with the following aims and objectives.

1) Collection of infected Plant leaves from Parbhani district
2) Isolation, purification and identification of *Colletotrichum spp.* causing leaf spot
3) Pathogenecity test.
4) Chemical control of leaf spot disease.
5) Biological control of leaf spot disease.

4) Materials and Methods :

1) Collection of infected plant leaves from different region of Parbhani District.
2) Isolation, Purification and Identification of different pathogenes.
3) Pathogenecity test.
4) Chemical control of leaf spot disease.
   i) Effect of different fungicides against *colletotrichum spp.*
5) Biological control of leaf spot disease.
   ii) Efficacy of different plant extract against *colletotrichum spp.*
6) Biocontrol agents.
   iii) Efficacy of different biocontrol agents against *colletotrichum spp.*

Future Prospectives

Traditions of Plant collection and Plant based medicines have been handed over from generation to generation. Plants collected from different sources shows wide disparity in therapeutic values in the recent years there has been industry in India. Consequently, the demand for the medicinal Plants has enormously increased. According to latest estimate there are about eight thousands liscensed pharmacies of Indian systems of medicine in the country engaged in the requirement of people. The total annual requirement of the raw materials of these pharmacies was estimated in tones. Presently, the increasing demand is fulfilled by cutting trees from their natural heritage and uprooting these trees / shoots leaves on nominal charges or by illegal cuttings.

This plant species has huge therapeutic potential; it has been over exploited by human activities. So, there is an urgent need to conserve it. Plant tissue culture techniques are the alternative method to rapid propagation of this plant for its conservation, for the enhancement of secondary product.

Recently, the phytodiversity is also being used by the scientists to produce nanoparticles. Green synthesis technology has been introduced for the synthesis of noble metallic nanoparticles by using Plant extract.

In such way, we will have opportunity to get benefited by the Plants. Hence further studies are required in the field of tissue culture. Mittal et al, (2014).

5) **Importance of Study :**

Tinospora cordifolia Plant is rich in many phytoconstituents i.e. useful in drug designing. Because of the vast immunological properties of Guduchi, phytochemical analysis of this plant is very useful in development new specialiazed drugs with more efficiency.

The pathogens infected to the leaf of *Tinospora cordifollia* causes heavy losses of leaf content therefore selected for detailed investigation in relation to *colletotrichum spp.*

6) Proposed work plan :

Sr. No.	Year	Duration	Proposed work
01	2018	Jan – Jun, 18	1) Course work and simultaneously Collection of leaf spot infected Plant leaves of *Tinospora cordifolia* from Parbhani district.
		Jun – Dec, 18	2) Isolation, purification and identification of *Colletotrichum spp.* 3) Pathogenicity test.
02	2019	Jan – Jun, 19	4) Chemical control of leaf spot diseases. i)Efficacy of different fungicides against *colletotrichum spp.*
		Jun – Dec,19	5) Biological control of leaf spot disease. ii) Effect of different plant extracts against *colletotrichum spp.*
03	2020	Jan – Jun,20	6) Biocontrol agents of leaf spot disease iii) Efficacy of different bio agents against *colletotrichum spp.*
		Jun – Dec,20	7) Finalization of obtained results, statistical analysis and submission of thesis.

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